On page 3, please delete the paragraph from line 7 to line 12 and insert the following rewritten paragraph:

This invention provides for the nucleic acid molecules that encode the proteins responsible for the thermal hysteresis in *Tenebrio* larvae. Nucleic acid sequencing predicts a thermal hysteresis protein (THP) having at least greater than one repeat of a 12 contiguous amino acid motif. This repeating motif is rich in cysteine and threonine (SEQ ID NO:1). In addition to the repeating motif, the N-terminus of the class of THP of this invention is a 16 amino acid motif (SEQ ID NO:3).

On page 5, please delete the paragraph from line 17 to line 22, and insert the following rewritten paragraph:

Figure 6 is an alignment chart of recombinant isoforms of THP (YL-1, YL-2, YL-4, YL-3 and 5-15 = SEQ ID NO:10, 12, 14, 16 and 18, respectively). The positions in which the nucleotide is conserved in all cDNA sequences (consensus = SEQ ID NO:20) are marked by an asterisk (*). The complete amino acid sequence is indicated only for YL-1 (SEQ ID NO:11). Residues of other isoforms (YL-2, YL-4, YL-3 and 5-15 = SEQ ID NO:13, 15, 17 and 19, respectively) which are identical to those found in YL-1 are indicated by a period (.). Differences are shown by boldface type where found. Gaps in both the cDNA and protein sequences are indicated by dashes (--------).

On page 6, please delete the paragraph from line 7 to line 21, and insert the following rewritten paragraph:

The primary structure of the mature THP is very unusual (Fig. 4) and is not similar to any other known sequence. The first 20 amino acids contain 6 Cys spaced at irregular intervals ($Cx_5Cx_2Cx_3Cx_2Cx_2C$; SEQ ID NO:22), and this sequence overlaps with the first of a series of 12-amino-acid repeats that continue until the end of the protein. Cys is repeated at 6-residue intervals throughout this region, which has the consensus

sequence CTxSxxCxxAxT (SEQ ID NO:1). The N-terminal Cys spacing has some elements in common with zinc-binding motifs (Klug &Schwabe, *FASEB J.* **9**:597 (1995)). However, extensive dialysis against 10 mM EDTA or 10 mM phenanthroline, and the subsequent addition of 2 mM $ZnCl_2$ (or 2 mM $CaCl_2$) to chelator-free preparations incubated for 1 h at 22°C does not affect activity, suggesting that there is no role for divalent metal ions in TH activity. At least some of the Cys residues are involved in disulfide bridges because all activity is lost on incubation with 10 mM dithiothreitol at 37°C for 20 min, whereas no activity is lost under the same conditions in the absence of reducing agent. There is no effect of N-ethylmaleimide on TH activity, which suggests that if free Cys are present they can be modified without loss of activity.

On page 9, please delete the paragraph from line 20 to line 24, and insert the following rewritten paragraph:

The term "contiguous amino acid motif" refers to a repeating pattern of amino acids present in a polypeptide or protein. The amino acids in each repeat do not have to be the same but there should be a pattern common to all. For example, in the class of proteins of the present invention, the repeating amino acid motif, cys-thr-xaa-ser-xaa-xaa cys-xaa-xaa-ala-xaa-thr (SEQ ID NO:1), where xaa is any amino acid, is present.

On page 16, please delete the paragraph from line 6 to line 12, and insert the following rewritten paragraph:

There are numerous methods for isolating the DNA sequences encoding the antifreeze protein of this invention. For example, DNA may be isolated from a genomic or cDNA library using labeled oligonucleotide probes having sequences complementary to the sequences or subsequences disclosed herein (SEQ ID NO:2 or 5). Such probes can be used directly in hybridization assays to isolate DNA encoding THP isoforms. Alternatively probes can be designed for use in amplification techniques such as PCR, and DNA encoding THP may be isolated by using methods such as PCR (see *infra*).

Please delete three paragraphs from page 17, line 29 to page 18, line 23, and insert the following rewritten paragraphs:

All of the above methods can be used to prepare DNA encoding antifreeze protein. In PCR techniques, oligonucleotide primers complementary to the two borders of the DNA region to be amplified are synthesized. The polymerase chain reaction is then carried out using the two primers (see Innis). In the instant invention, because of the presence of repetitive motifs, the length of the THP subsequence encoded by the amplified product will depend on the template used. Because the N and C termini are unique (*i.e.,* different nucleotide sequences from the repetitive motif), to amplify the full-length THP encoding sequence, the primers of SEQ ID NO: 6 and 7 can be used.

PCR can be used in a variety of protocols to isolate nucleic acids encoding partial sequences of THP. In these protocols, appropriate primers and probes for amplifying DNA encoding partial sequences of THPs are generated from analysis of the DNA sequences listed herein. For example, the oligonucleotides of SEQ ID NO:6, 7, 8 and 9 can be used in a PCR protocol to amplify regions of DNA which encodes THPs. Once such regions are PCR-amplified, they can be sequenced and labeled oligonucleotide probes can be prepared from the sequence obtained. These probes can then be used to isolate DNA encoding the complete THP from DNA libraries.

SEQ ID NO:2 and 5 represent isoforms of naturally occurring *Tenebrio molitor* THP cDNA. They are not complete DNA gene sequences. However, the partial antifreeze nucleic acid sequence of SEQ ID NO:2 or 5 can be completed according to standard methods well known to those of skill in the art. A preferred approach for DNA isolation is RACE. Briefly, this technique involves using PCR to amplify a cDNA sequence using a random 5' primer and a defined 3' primer (5' RACE) or a random 3' primer and a defined 5' primer (3' RACE). The amplified sequence is then subcloned into a vector where it is then sequenced using standard techniques. The RACE method is well known to those of skill in the art and kits to perform RACE are commercially available (*e.g.,* 5' RACE System, GIBCO BRL, Grand Island, New York, USA).

On page 45, please delete the paragraph from line 7 to line 12, and insert the following

rewritten paragraph:

In a particularly preferred embodiment, nucleic acid sequences comprising the structural gene in question or upstream sequences are utilized for targeting heterologous recombination constructs. Utilizing the structural gene sequence information provided in SEQ ID NO:2 and 5 or the upstream or downstream sequence information provided in SEQ ID NO:10, 12, 14 and 16, one of skill in the art can create homologous recombination constructs with only routine experimentation.

Please delete the two paragraphs on page 48, line 22 to page 49, line 2, and insert the following rewritten paragraphs:

To isolate other isoforms of the THP of this invention, oligonucleotides for probes (SEQ ID NO:2 and 5), PCR primers (SEQ ID NO:6 and 7) and sequencing primers SEQ ID NO: 8 and 9) were designed based on the consensus sequence determined from sequencing TH positive clones (YL-1-4).

Aliquots of a *Tenebrio molitor* larval fat body λ-Zap cDNA library (see, Graham, *et al., Insect Biochem. Molec. Biol.* **26**:127 (1996)) were screened with the nucleic acid sequence of YL-1 from the 5' end to the stop codon (SEQ ID NO:21). Approximately 1 x 10^5 plaques were screened at moderate stringency following standard methodologies using the sequence listed above. Isolated positive plaques were subjected to *in vivo* excision using R408 helper phage (Stratagene) as per manufacturer's instructions. The double-stranded DNA obtained was purified and sequenced as above using the vector primers T7 and T3 as well as SEQ ID NO:8 and 9.

Please cancel the four pages containing SEQ ID NO:1 through SEQ ID NO:16 currently on file, and substitute therefor the accompanying paper copy of the Sequence Listing, pages 1 to 14.

IN THE CLAIMS:

Please cancel claims 1 to 35, and enter new claims 36 to 81 as follows: